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Reverse-phase high performance liquid chromatography separation of positional isomers on the MIL-53(Fe) packed column †

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Metal-organic framework (MOFs) MIL-53(Fe) applied as a novel stationary phase for reverse-phase high performance liquid chromatography (HPLC) separation of positional isomers was described for the first time. Under the optimized conditions, baseline separation of xylene, dichlorobenzene, chlorotoluene and nitroaniline isomers was achieved on the MIL-53(Fe) packed column within short time. The retention mechanisms of these isomers on the MIL-53(Fe) packed column were discussed in detail, and a typical reversed-phase behavior can respond to the results. The thermodynamic charatcers of HPLC separation of positional isomers were also evaluated. It was confirmed that HPLC separation of positional isomers was controlled by Gibbs free energy change (ΔG). In addition, reverse-phase HPLC separation of the tested isomers were also carried on the MIL-53(Al, Cr) packed columns to investigate the influence of metal centre of MIL-53 framework on their chromatographic behaviors. The results showed that the MIL-53(Fe) packed column exhibited better separation performance than the MIL-53(Al, Cr) packed columns. Moreover, the MIL-53(Fe) packed column also gave efficient HPLC separation of alkylbenzene and naphthalene, aniline compounds and polycyclic aromatic hydrocarbons (PAHs). The successful applications suggested the potentials of MIL-53(Fe) as a novel stationary phase for efficient reverse-phase HPLC separation.

1. Introduction

Substituted aromatic positional isomers are widely used in the fields of organic intermediates, pesticides, medicines and dyestuff. Meanwhile, many of substituted aromatic positional isomers are poisonous compounds, which are harmful to human health and environment. Therefore, separation and analysis of substituted aromatic positional isomers is of vital important for manufacturing monitoring and environmental analysis. Since the chemical and physical properties of positional isomers are very similar, efficient separation of positional isomers remains a big challenge. The commonly used C8 or C18 columns are not very suitable for separation of positional isomers based on hydrophobic interaction.[1] To date, considerable efforts have been devoted to develop novel stationary phase, there are some types of stationary phases available for separation of positional isomers, including modified zirconia and calixarenes bonded silica stationary phase.[2-7] Although these stationary phases have achieved some success, additional stationary phases developed for separation of positional isomers are still required.

Metal-organic framewoks (MOFs), a new class of nanoporous crystalline materials, which are constructed from clusters or chains of metal ions connected by organic ligands to create one-, two-, and three dimensional porous structures.[8] The fascinating properties of MOFs, such as high surface area, the availability of modification their pore surface, active metal site, good thermal and chemical stability,[9-11] make it a suitable stationary phase for gas chromatography (GC) and HPLC separation.[12-33] Because of their functional internal surface, MOFs used as stationary phase can provide various retention mechanisms, including π-π stacking, hydrogen bonding and coordination with open metal sites,[34] which are very suitable for separation of positional isomers. Recently, several MOFs and MOFs composites have been explored as stationary phases for HPLC separation of positional isomers.[19, 20, 24, 29, 35-39] However, most of the MOFs packed HPLC columns used for separation of positional isomers were carried out in normal-phase HPLC, even though reverse-phase HPLC is the most popular analysis technique used in the field of biochemistry and chemistry. To the best of our knowledge, only MIL-53(Al), MIL-100(Fe) and UiO-66 had been explored as stationary phases for reverse-phase separation of positional isomers.[32, 38-40] Therefore, development of novel MOFs packed column used for separation of positional isomers in reverse-phase HPLC is highly desirable.

MIL-53(Fe) is one of the well known group of MIL-53 MOFs, which are built up from corner-sharing metal (Al, Cr, Fe, Ga, In, V) clusters interconnected with benzenedicarboxylate organic ligands to form a one-dimensional lozenge-shaped pore channel system.[41] The MIL-53 framework has structural flexibility or 'breathing', they could adapt their pore size according to the guest species without a loss of crystallinity or bond breaking. This extraordinary property of MIL-53 material makes them suitable for use in the field of liquid phase separation and adsorption. Previous studies have showed that MIL-53(Al) could be used as stationary phase for both normal-phase and reverse-phase HPLC separation of positional isomers.[19, 20, 40] In normal phase HPLC, the MIL-53(Al) packed column showed high separation ability for substituted aromatic positional isomers. In reverse phase HPLC, the MIL-53(Al) packed column only gave long separation time for benzenediol isomers. MIL-53(Fe) has also been explored in liquid separation of mixture of BTEX (benzene, toluene, ethylbenzene and the three xylene isomers),[42] but systematic studying on its application in reverse phase HPLC has yet not been carried out as far as we know. On the other hand, it has been found that the metal center of MOFs greatly affected their performance in chromatographic separation. For example, Fan and Yan observed that capillary columns

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coated with MIL-100(Fe) or MIL-100(Cr) exhibited different GC separation performances for alkane isomers.[43] Based on the above aforementioned points, it is still worthy to investigate the reverse-phase HPLC performance of MIL-53(Fe), with the possibility to develop high selective stationary phase that may outperform existing materials.

Herein, we report MIL-53(Fe) as a novel stationary phase, which can give high-resolution separation for positional isomers in reverse-phase HPLC. Four types of positional isomers (xylene, nitroaniline, chlorotoluene and dichlorobenzene isomers) were used to investigate reverse-phase HPLC separation of positional isomers on the MIL-53(Fe) packed column using acetonitrile (ACN)/H_2O as the mobile phase. The separation performance of positional isomers on the MIL-53(Fe) packed column was also compared to MIL-53(Al, Cr) packed columns, commercial C8 and C18 columns, respectively. In addition, alkylbenzenes, aniline compounds and polycyclic aromatic hydrocarbons (PAHs) were also separated on the MIL-53(Fe) packed column to evaluate the potentials of MIL-53(Fe) in reverse-phase HPLC applications besides separation of positional isomers.

2. Experimental

2.1. Materials

All chemicals and reagents used were analytical grade or better. Xylene, ethylbenzene (EB), toluene, ACN, dichlorobeneze， chlorotoluene, nitroaniline, iron(III) chloride hexahydrate, aluminum nitrate nonahydrate, chromium(III) nitrate nonahydrate and hydrofluoric acid were purchased from Sinoparm Chemical Reagent Co. Ltd. (Shanghai, China). Methanol and N,N-dimethylformamide (DMF) were obtained from Fuchen Chemical Reagents Factory (Tianjin, China). Terephthalic acid was the product of Aladdin Chemistry Co. Ltd. (Shanghai, China). Ultrapure water was deionized in a Milli-Q SP reagent water system (Millipore, Bedford, MA, USA).

2.2. Apparatus

All chromatographic experiments were performed on an Agilent 1100 HPLC system equipped with a 20 μL sample loop, a G1379 degasser, a G1311 quaternary solvent delivery system and a G1314 variable wavelength detector. C8 column (25cm × 4.0mm i.d., 5 μm) was obtained from Agilent Technologies Inc. (ZORBAX SB-C8, Agilent, USA). C18 column was the product of Bischoff Chromatography (25cm × 4.0mm i.d., 5 μm) (ProntoSIL Eurobond, Germany). Scanning electron micrograph (SEM) images were carried out on XL30E scanning electron microscope (Philips, The Netherlands). The thermogravimetric analysis (TGA) over the temperature ranging from room temperature to 600 ℃ (heating rate of 10 ℃) was performed in a STA449C thermogravimetric analyzer (Netzsch, German) under the inert atmosphere (Ar). A X'Pert Pro MPD diffractometer (Philips, Netherlands) was used to record the X-ray diffraction (XRD) patterns using Co Kα radiation (λ= 1.789 Å). The surface area was determined on an ASAP 2020 (Micromeritics, USA) at 77 K using nitrogen adsorption.

2.3. Synthesis of MIL-53

MIL-53(Fe) was hydrothermally synthesized according to Millange et al. with some modifications.[44] Briefly, hexahydrated iron chloride (10 mmol), terephthalic acid (10 mmol) were mixed with N,N'-dimethylformamide (DMF, 50 mL). Reactants were stirred for a few minutes before introducing the resulting suspension in a Teflon-lined steel autoclave and the temperature was set at 423 K for three days. After cooling down, the light orange MIL-53(Fe) solid was washed thoroughly with deionized water and drying in air.

MIL-53(Al) was hydrothermally synthesized according to Loiseau et al.[45] Typically, 6500 mg of aluminum nitrate nonahydrate and 1440 mg of terephthalic acid were mixed with 25 mL of ultrapure water in a Teflon-lined stainless steel autoclave, which was heated at 493 K for 3 days. After cooling down, the white powder obtained was washed with ultrapure water. The product obtained was reheated at 553 K to remove terephthalic acid residues.

MIL-53(Cr) was hydrothermally synthesized according to Ferey et al.[46] Typically, a mixture of chromium (III) nitrate, terephthalic acid, hydrofluoric acid, and H_2O in the molar ratio 1:1:1:280 was heated at 493 K for 3 days. The solid obtained was washed four times in 200 mL ethanol at 343 K to remove terephthalic acid residues.

2.4. Preparation of the MIL-53(Fe) packed column for HPLC

A 1.5 g mass of MIL-53(Fe) was dispersed in MeOH (10 mL) under ultrasonication for 10 min. The suspension was then packed into a stainless steel column (200 mm × 2.1 mm i.d.) under 35 MPa using the slurry technique. The MIL-53(Fe) packed column was washed with methyl alcohol for 2 h before use. The MIL-53(Al) and MIL-53(Cr) packed columns were prepared by the same procedure as prepared for the MIL-53(Fe) packed column.

2.5. Calculation of thermodynamic parameters

The Enthalpy change (ΔH), entropy change (ΔS) and Gibbs free energy change (ΔG) for the transfer of solutes from the mobile phase to the stationary phase MIL-53(Fe) were calculated from the following van't Hoff equation.[21]

$$\ln k = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} + \ln \Phi \quad (1)$$

$$\Delta G = \Delta H - T\Delta S \quad (2)$$

where k is the retention factor, R is the gas constant, T is the absolute temperature and Φ is the phase ratio. The k value was calculated according to equation (3):

$$k = (t - t_0)/t_0 \quad (3)$$

where t is the retention time, t_0 is the column void time which was determined by injecting a small plug of acetonitrile and recording the perturbation signal.

3. Results and discussion

3.1. Characterization

The synthesized MIL-53(Fe) was characterized by XRD, SEM and TGA. The XRD pattern showed that the diffraction peaks of the synthesized MIL-53(Fe) were in good agreement with the simulated one and no impurity peaks were detected in the synthesized MIL-53(Fe), indicating the successful preparation of MIL-53(Fe) (Fig.1A). The TGA data showed that the MIL-53(Fe) was stable up to 350°C and has two weight loss (Fig.1B), which can be attributed to surface water evaporation (< 100°C) and collapse of structure of MIL-53(Fe) (350-500°C). The synthesis MIL-53(Fe) crystals show cubic shape with a broad size distribution (Fig.1C). The Brunauer–Emmett–Teller (BET) surface area and pore volume of MIL-53(Fe) were calculated to

be 19 m^2 g^{-1} and 0.013 m^3 g^{-1}, respectively. The very low surface area of MIL-53(Fe) was attributed to the fact that the anhydrous form of MIL-53(Fe) exhibits closed pore with no accessible porosity to most gases.[47]

The stability of MOFs plays an important role in the application of MOFs as HPLC stationary phase. In order to investigate the mechanical stability of the MIL-53(Fe) packed column, the pressure drop across the column was measured in different flow-rates and mobile phases. Fig.1D showed the effect of flow rate and mobile phase on the back pressure. An excellent linear dependence of the column pressure on the flow rate and mobile phase was indicated by goodness of fit (R^2) better than 0.99 for the measured curve. In addition, no significant change in the particle size distribution was observed after chromatographic experiments (Fig.S1 in Supplementary materials). The above results indicated that MIL-53(Fe) exhibited good stability for HPLC analysis. It also can observed that the the MIL-53(Fe) packed column gave relatively low back pressure, the maximum backe pressure was only 130 bar at a flow rate of 0.6 mL/min using 50% ACN as the mobile phase, which was attractive for practical HPLC application.

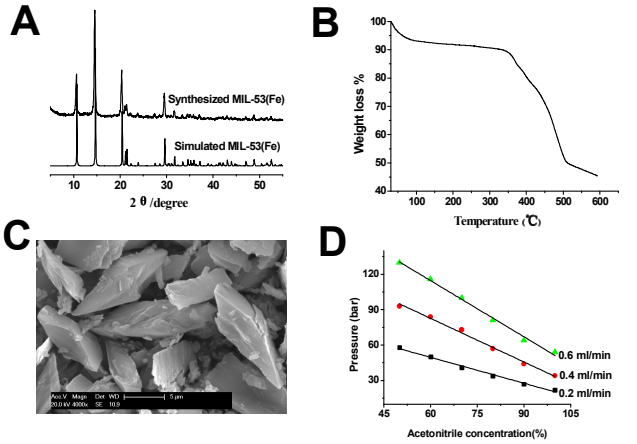

Fig. 1 (A) XRD patterns of MIL-53(Fe) and Simulated MIL-53(Fe); (B) TGA of MIL-53(Fe); (C) SEM of MIL-53(Fe); (D) Dependence of the back pressure of MIL-53(Fe) packed column on the flow-rate and mobile phase.

3.2 HPLC separation of positional isomers on the MIL-53(Fe) packed column

3.2.1 HPLC separation of xylene isomers

The separation of xylene isomers remains a challenge because of their close chemical and physical properties. To date, several MOFs have been reported good selectivity for these positional isomers in normal-phase HPLC. For comparison, xylene isomers were used to test the characteristic of reverse-phase HPLC separation of positional isomers on the MIL-53(Fe) packed column. It can be seen from Fig.2A that xylene isomers can be well separated within 4 minutes using 100% ACN as the mobile phase at a flow rate of 0.6 mL min^{-1}. The retention sequence of xylene isomers followed an increasing order of p-xylene < o-xylene < m-xylene, depending on molecular stacking effects and hydrophobic interaction between xylene isomers and MIL-53(Fe). Both m-xylene and o-xylene have been reported to stack pairwise in the MIL-53(Fe) pores and have a close distance and strong π-π stacking interaction with the inside pore walls.[42] While p-xylene molecules are arranged in a zig-zag manner rather than in pairs, resulting in ineffective interactions with the terephthalate linkers of the adjacent wall and fast eluted from the MIL-53(Fe) packed column. In addition, m-xylene eluted after before o-xylene on the MIL-53(Fe) packed column HPLC mainly caused by the greater hydrophobic interaction provided by the higher hydrophobicity of m-xylene than o-xylene, showing the reverse-shape characteristic of the MIL-53(Fe) packed column.

3.2.2 HPLC separation of chlorotoluene and dichlorobenzene isomers

Normal-phase HPLC separation of chlorotoluene and dichlorobenzene isomers had been explored on the $Cu_3(BTC)_2$, MIL-53(Al) and MIL-101(Cr) packed columns with poor separation efficiency or long separation time.[19, 29, 48] In this work, dichlorobenzene isomers can be baseline separated on the MIL-53(Fe) packed column using ACN/H_2O (80:20) as the mobile phase at a flow rate of 0.6 mL min^{-1} (Fig. 2B). The R_S values for o-/p- dichlorobenzene and m-/p- dichlorobenzene were 1.86 and 2.04, respectively. The MIL-53(Fe) packed column also gave fast separation of chlorotoluene (Fig. 2C) using 100% ACN as the mobile phase at a flow rate of 0.6 mL min^{-1}. The R_S values for o-/p- chlorotoluene and m-/p- chlorotoluene were 2.45 and 1.4, respectively. The elution orders of chlorotoluene and dichlorobenzene isomers followed an increasing order of para-isomers < ortho-isomers < meta-isomers, the same as the order of xylene isomers, suggesting that the retention mechanisms of chlorotoluene and dichlorobenzene isomers on the MIL-53(Fe) packed column can be explained similarly to that of xylene isomers. In addition, it can be found that the retention times of chlorotoluene and dichlorobenzene isomers were very high in comparison to that of xylene isomers (Fig. S2 in Supplementary materials), which was attributed to π-electron transfer interaction resulting from the electron-withdrawing effect of the chloride group of solutes and electron-releasing effect of the hydroxyl groups of MIL-53(Fe).

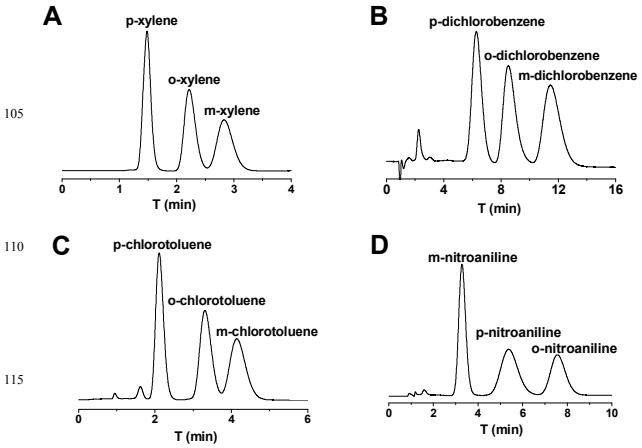

Fig. 2 Chromatograms for HPLC on MIL-53(Fe) packed column: (A) xylene isomers using ACN/H_2O (100:0) as the mobile phase; (B) dichlorobenzene isomers using ACN/H_2O (80:20) as the mobile phase; (C) chlorotuluene isomers using ACN/H_2O (100:0) as the mobile phase; (D) nitroanline isomers using ACN/H_2O (70:30) as the mobile phase at a flow rate of 0.6 mL min^{-1}. All the separations were performed at room temperature and monitored with a UV detector at 254 nm.

3.2.3 HPLC separation of nitroaniline isomers

Nitroaniline isomers are strongly toxic and widely considered as potential carcinogenic substances, nitroaniline isomers have been included in the list of priority pollutants in some countries. Therefore separation and determination of nitroaniline isomers are important for environmental analysis. As shown in Fig.2D, nitroaniline isomers can be baseline separated on the MIL-53(Fe) packed column using ACN/H_2O (70:30) as the mobile phase at a flow rate of 0.6 mL min^{-1}. The R_S values for p-/m-nitroaniline and o-/p-nitroaniline were 2.26 and 1.8, respectively. The elution

orders of nitroaniline isomers followed an increasing order of m- < p- < o-nitroaniline. The favorable affinity of MIL-53(Fe) for o-nitroaniline may be ascribed to the higher hydrophobic interaction provided by the intramolecular hydrogen bonding of o-nitroaniline and its lowest dipole moment (4.38 D) among the three isomers, which showed the reverse-phase properties of MIL-53(Fe) packed column. In addition, the elution orders of nitroaniline isomers was in accordance with the order of their pK_b values (the pK_b values of m-nitroaniline, p-nitroaniline and o-nitroaniline are 11.4, 12.9 and 14.2, respectively), suggesting that the retention of nitroaniline isomers may be also controlled by the nature of the nitro group. The electronic cloud density of the nitro group for m-, p- and o-nitroaniline increased as the pK_b increased, thus the hydrogen bonding interaction between the nitro group of nitroaniline and the hydroxyl group of MIL-53(Fe) increased with an increasing order of m-, p-, o-nitroaniline.

3.2.4 Effect of mobile phase on HPLC separation

To demonstrate the reverse-phase separation mechanism on the MIL-53(Fe) packed column, the effect of mobile phase on HPLC separation of positional isomers was investigated by changing the proportions of ACN in the mobile phases (Fig.S2 in Supplementary materials). The relationship of log *k* of solutes and ACN content of the mobile phase on MIL-53(Fe) packed column was studied and shown in Fig.3. It is obvious that the log *k* of xylene, chlorotoluene and dichlorobenzene isomers decreased with the increasing of ACN content, which illustrated that the hydrophobic interaction between solutes and MIL-53(Fe) was the main factor for the retention of xylene, chlorotoluene and dichlorobenzene isomers on the MIL-53(Fe) packed column. Similar phenomenon was also found for nitroanline isomers as the content of ACN decreased from 90 to 70%, indicating that hydrophobic interaction played an important in the separation of nitroanline isomers. However, the mobile phase has different influences on the retention factor of p-, m- and o-nitroanline as the content of ACN decreased from 100 to 90% (Fig.3D), which implied that there are strong secondary interactions between nitroanline isomers and MIL-53(Fe) besides hydrophobic interaction at the ACN content higher than 90%.

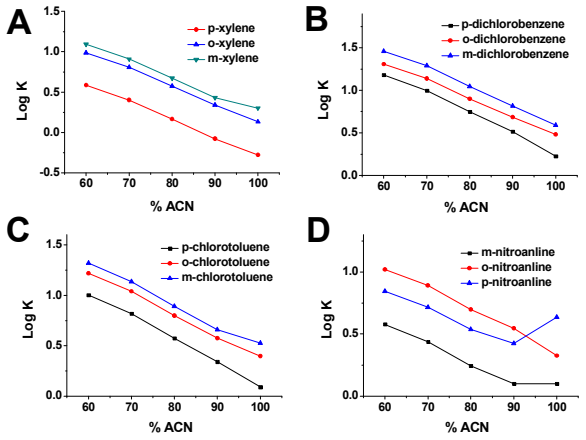

Fig. 3 Effect of the ACN content of mobile phase on retention factors of positional isomers on the MIL-53(Fe) packed column at a flow rate of 0.6 mL min^{-1}: (A) xylene isomers; (B) dichlorobenzene isomers; (C) chlorotuluene isomers; (D) nitroanline isomers. All the separations were performed at room temperature and monitored whith a UV detector at 254 nm.

It was also observed that the mobile phase significantly affected the resolution of the solutes on the MIL-53(Fe) packed column (Table.S1 in Supplementary materials). Taking xylene isomers as an example, the HPLC resolution of xylene isomers decreased as the ACN content in the mobile phase decreased from 100 to 90%, and then increased gradually as the content of ACN decreased from 90 to 60%. The above results revealed that the mobile phase played a significant role in the HPLC separation of substituted aromatic positional isomers on the MIL-53(Fe) packed column.

3.2.5 Effect of temperature on HPLC separation

To investigate the thermodynamic properties of the HPLC separations on MIL-53(Fe) column, six temperatures (25, 35, 45, 55, 65 and 75 °C) were selected to investigate the separation performances of xylene, dichlorobenzene, chlorotoluene and nitroaniline isomers. It was found that as the temperature increased, changes of the retention times showed different trends for different aromatic isomers (Fig.4). The retention of m-xylene decreased as the temperature increased from 25-35°C and then kept no changes with further increased the temperature, whereas the retention of m-dichlorobenzene, m-chlorotoluene and p-nitroaniline decreased gradually as the temperature increased. On the other hand, the retention of other isomers gradually increased as the temperature increased. Accordingly, it is found that the MIL-53(Fe) packed column gave high selectivity for m-isomers of xylene, dichlorobenzene and chlorotoluene isomers at 25°C, but gave high selectivity for o-isomers of xylene, dichlorobenzene and chlorotoluene isomers at 75°C. Also, the selectivity of the aromatic isoemrs changed significantly as the temperature increased, indicating the feasibility of realizing separation on MIL-53(Fe) packed column via controlling temperature (Table.S2 in Supplementary materials).

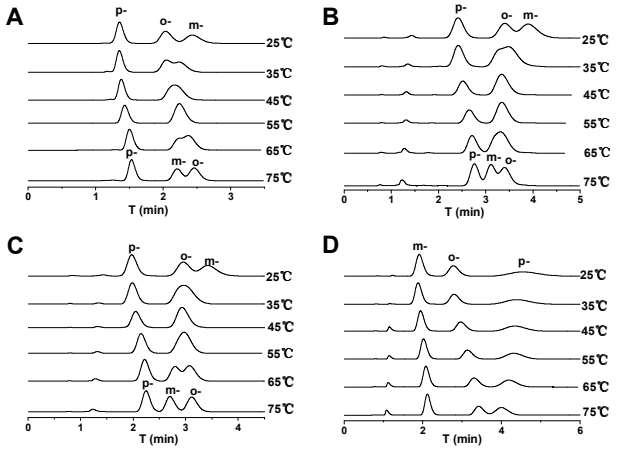

Fig.4 Effect of temperature on the HPLC chromatograms on the MIL-53(Fe) packed column for the separation of: (A) xylene isomers, (B) dichlorobenzene isomers, (C) chlorotuluene isomers and (D) nitroanline isomers at 25-75 °C using 100% ACN as the mobile phase at a flow rate of 0.6 mL min^{-1}. All the signals were monitored with a UV detector at 254nm.

The van't Hoff equation was used to demonstrate the relationship between retention factor and column temperature. As shown in Fig. 5, ln *k* was linearly related to 1/*T*, indicating that no changes in the interaction mechanism in the temperature range of 25-75 °C for the separation of xylene, dichlorobenzene, chlorotoluene and nitroaniline isomers in RP-HPLC. The values of ΔH and ΔS obtained from the van't Hoff plot were summarized in Table 1, and those of ΔG at different temperature were summarized in Table 2. The transfer of m-dichlorobenzene, m-chlorotoluene and p-nitroaniline from mobile phase to MIL-53(Fe) was controlled by negative ΔH and positive ΔS, whereas other isomers were controlled by positive ΔH and ΔS. The results also implied that separation of these isomers was not controlled by ΔH or ΔS individually, but both of them simultaneously. The values of ΔG were also used to evaluate the transfer of analytes from mobile phase to MIL-53(Fe). The negative of ΔG for these

isomers indicated that the transfer of these isoers from the mobile phase to MIL-53(Fe) was thermodynamically spontaneous. Additionally, it is clearly found that more negative ΔG correlate with a more favorable transfer of the solute from the mobile phase to MIL-53(Fe), thus stronger retention for the solute on MIL-53(Fe).

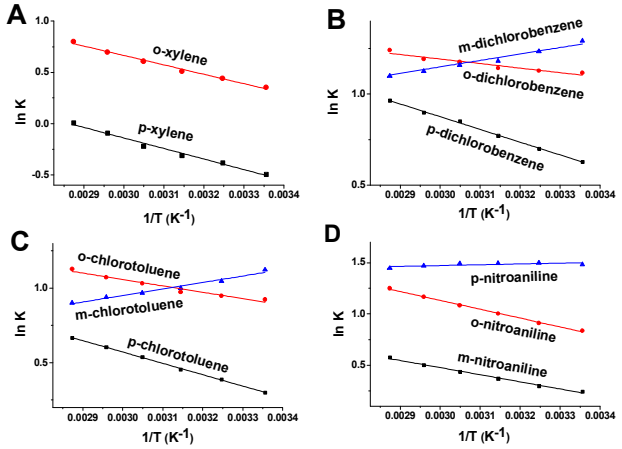

Fig.5 Van't hoff plot for: (A) xylene isomers, (B) dichlorobenzene isomers, (C) chlorotuluene isomers and (D) nitroanline isomers on the MIL-53(Fe) packed column. Separation conditions as shown in Fig.4.

Table1. Values of ΔH, ΔS and R^2 for xylene, dichlorobenzene, chlorotoluene and nitroaniline isomers.

Analytes	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} k^{-1})	R^2
p-xylene	8.87	33.64	0.97783
o-xylene	8.03	37.80	0.98718
p-chlorotoluene	6.29	31.81	0.99799
o-chlorotoluene	4.07	29.19	0.97485
m-chlorotoluene	-2.56	8.48	0.93483
p-dichlorobenzene	5.84	32.99	0.99553
o-dichlorobenzene	2.44	25.40	0.92853
m-dichlorobenzene	-2.44	10.41	0.90128
m-nitroaniline	6.15	30.60	0.99708
o-nitroaniline	7.5	40.09	0.9992
p-nitroaniline	-1.12	17.06	0.91210

3.3 Advantages of MIL-53(Fe) packed columns over MIL-53(Al, Cr) packed columns and commercial columns for the separation of positional isomers

The separation performance of MIL-53(Fe) packed column was compared with MIL-53(Al, Cr) packed columns. The MIL-53(Al) packed column offered good separation of p-, m- and o-xylene within 3 min using pure ACN as the mobile phase (Fig.S3 in Supplementary materials). However, the MIL-53(Al) packed column was unable to discriminate ortho and para isomers for separation of chlorotoluene, dichlorobenzene and nitroaniline isomers using pure ACN as the mobile phase. The MIL-53(Cr) packed column also gave a poor separation performance since the elution time of the tested isomers was very long caused by peak broadening and tailing peak (Fig.S4 in Supplementary materials). The above results showed obvious advantages of the MIL-53(Fe) packed column over MIL-53(Al, Cr) packed columns. In addition, it was also found that the elution order of the tested isomers was different on the MIL-53 packed columns. For example, the retention sequence of xylene isomers on the MIL-53(Al, Cr) packed columns in reverse-phase HPLC followed an increasing order of p-xylene < m-xylene < o-xylene, which was not the same as the order on MIL-53(Fe) packed column. The above results demonstrated that the metal center of MIL-53 played an important role in the HPLC separation of xylene, dichlorobenzene, chlorotoluene and nitroaniline isomers.

Table2. Values of ΔG for xylene, dichlorobenzene, chlorotoluene and nitroaniline isomers.

Analytes	ΔG					
	25°C	35°C	45°C	55°C	65°C	75°C
p-xylene	-1.15	-1.49	-1.82	-2.16	-2.5	-2.83
o-xylene	-3.23	-3.61	-3.99	-4.37	-4.75	-5.12
p-chlorotoluene	-3.19	-3.51	-3.82	-4.14	-4.46	-4.78
o-chlorotoluene	-4.63	-4.92	-5.21	-5.5	-5.8	-6.09
m-chlorotoluene	-5.09	-5.17	-5.26	-5.34	-5.43	-5.51
p-dichlorobenzene	-3.99	-4.32	-4.65	-4.98	-5.31	-5.64
o-dichlorobenzene	-5.13	-5.39	-5.64	-5.9	-6.15	-6.4
m-dichlorobenzene	-5.54	-5.65	-5.75	-5.86	-5.96	-6.07
m-nitroaniline	-2.97	-3.27	-3.58	-3.88	-4.19	-4.5
o-nitroaniline	-4.45	-4.85	-5.25	-5.65	-6.05	-6.45
p-nitroaniline	-6.21	-6.38	-6.55	-6.72	-6.89	-7.06

Commercial C8 and C18 columns were also employed for comparison to highlight the outstanding separation performance of MIL-53(Fe) packed column. C8 and C18 columns are the most commonly used silica bonded columns for reverse-phase HPLC separation. However, both C8 and C18 columns exhibited poor selectivity for separation of xylene, chlorotoluene and dichlorobenzene isomers (Fig.S5, S6 and Table.S3 in Supplementary materials). In contrast, the MIL-53(Fe) packed column exhibited good selectivity for separation of these isomers (Table.S2 in Supplementary materials), revealing the advantages of the MIL-53(Fe) packed column over commercial C8 and C18 columns for the separation of xylene, chlorotoluene and dichlorobenzene isomers. Nevertheless, the MIL-53(Fe) packed column faced the problem of low column efficiency by comparing the separation of nitroaniline isomers on MIL-53(Fe) packed column and C18 column (Table.S4 in Supplementary materials). Further research should pay more attention to improve column efficiency of MIL-53(Fe) packed column.

3.4 Performance of MIL-53(Fe) packed column for the HPLC separation of the positional isomers

The MIL-53(Fe) packed column gave good reproducibility (Fig.S7 in Supplementary materials) for reverse-phase HPLC separation of xylene, chlorotoluene, dichlorobenzene and nitroaniline isomers. The relative standard deviation (RSD) of retention time, peak area, peak height and half peak width for five replicate separations of the tested isomers were 0.09-0.18%, 0.07-0.21%, 0.06-0.17%, and 0.05-0.18%, respectively (Table 3). The column-to-column reproducibility for the preparation of MIL-53(Fe) packed columns were also evaluated (Fig.S8 in Supplementary materials). The low RSD values of column-to-column indicated the reliability of MIL-53(Fe) as a stationary phase (Table.S5 in Supplementary materials). Besides, an increase in analyte mass resulted in a linear increase of the chromatographic peak area (Figure.S9). These features of the MIL-53(Fe) packed column make it a promising candidate as the stationary phase for reverse-phase HPLC separation of positional isomers.

3.5 Applications of the MIL-53(Fe) packed column

In order to extend the potential of MIL-53(Fe) in reverse-phase HPLC applications besides separation of positional isomers, three groups of analytes, that is alkylbenzene and naphthalene, aniline compounds and polycyclic aromatic hydrocarbons (PAHs) were

Table3. Precision for five replicate separations on the MIL-53(Fe) packed column

Analytes	RSD (%) (n=5)			
	Retention time	Peak area	Peak high	Half peak width
p-xylene	0.11	0.14	0.1	0.05
m-xylene	0.13	0.09	0.11	0.12
o-xylene	0.09	0.14	0.13	0.08
p-dichlorobenzene	0.09	0.12	0.14	0.10
m-dichlorobenzene	1.15	0.13	0.09	0.08
o-dichlorobenzene	0.13	0.10	0.08	0.09
p-chlorotoluene	0.10	0.07	0.06	0.12
m-chlorotoluene	0.12	0.16	0.10	0.08
o-chlorotoluene	0.18	0.20	0.12	0.09
p-nitroaniline	0.16	0.14	0.11	0.10
m- nitroaniline	0.12	0.21	0.13	0.13
o- nitroaniline	0.15	0.2	0.17	0.18

also separated on the MIL-53(Fe) packed column. Effective separation of neutral alkylbenzene and naphthalene could be achieved on the MIL-53(Fe) packed column (Fig.6A). As it can be seen from the results, the retention time of benzene, toluene, ethylbenzene and naphthalene decreased with the increase of ACN content, confirming again that the hydrophobic interaction played a dominant role in the separation of the alkylbenzene and naphthalene. In addition, the hydrophobicity strength of analytes follows the order of benzene < toluene < ethylbenzene < naphthalene, while ethylbenzene was eluted earlier than toluene on the MIL-53(Fe) packed column. The column gave stronger retention for toluene than ethylbenzene, which may be attributed to the fact that ethyl group has weaker interaction with carboxylate moieties in the obtuse pore corners than methyl group due to steric effects.[20]

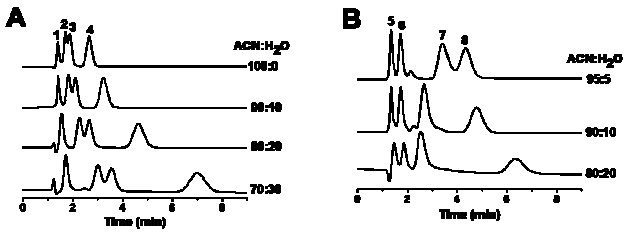

Fig.6 HPLC chromatograms on the MIL-53(Fe) packed column for the separation of (A) benzene (1), ethylbenzene (2), toluene (3), naphthalene(4); (B) diphenylamine (5), aniline (6), 1,2-diaminobenzene (7), 1-naphthylamine (8) using different ratios of ACN/H_2O at a flow rate of 0.4 mL min^{-1}. All the separations were performed at room temperature and monitored whith a UV detector at 254 nm.

Four kinds of basic aniline compounds were also well separated on the MIL-53(Fe) packed column (Fig.6B). The retention time of basic aniline followed an increasing order of diphenylamine < aniline < 1,2-diaminobenzene < 1-naphthylamine. Diphenylamine has the largest kinetic diameter among the four compounds and it was eluted first on the MIL-53(Fe) packed column regardless of the composition of the mobile phase as a result of size exclusion. The MIL-53(Fe) packed column showed highest affinity for 1-naphthylamine, which was attributed to the hydrogen bond interaction and the strong π–π stacking interaction between 1-naphthylamine and MIL-53(Fe). Compared with aniline, 1,2-diaminobenzene was strongly retained on the MIL-53(Fe) packed column, suggesting an additional interaction between the amino group of 1,2-diaminobenzene and the hydroxyl group of MIL-53(Fe).

The MIL-53(Fe) packed column also showed satisfactory separation performance for PAHs (Fig.7). The six PAHs were eluted in order of naphthalene < acenaphthylene < benzo(k)fluoranthene < anthracene < fluorine < phenanthrene, which is not in accordance with both the hydrophobic interaction and π–π stacking interaction between the PAHs and MIL-53(Fe), indicating that the retention of PAHs on the column may be a composite result from various retention interactions.

4. Conclusions

In conclusion, we have demonstrated MIL-53(Fe) as a promising stationary phase for reverse-phase HPLC separation of positional isomers. The MIL-53(Fe) packed column provided a diverse range of host-guest interactions including hydrogen bond interaction, hydrophobic interaction, π–π stacking effect and size exclusion. Based on various retention mechanism, xylene, dichlorobenzene, chlorotoluene and nitroaniline isomers can be separated on the MIL-53(Fe) packed column in reverse-phase HPLC with good selectivity and reproducibility. In addition, the MIL-53(Fe) packed column exhibited better separation performance of these positional isomers than MIL-53(Al, Cr), C8 and C18 packed columns. Furthermore, the MIL-53(Fe) packed column also gave efficient HPLC separation of alkylbenzene and naphthalene, aniline compounds and PAHs.

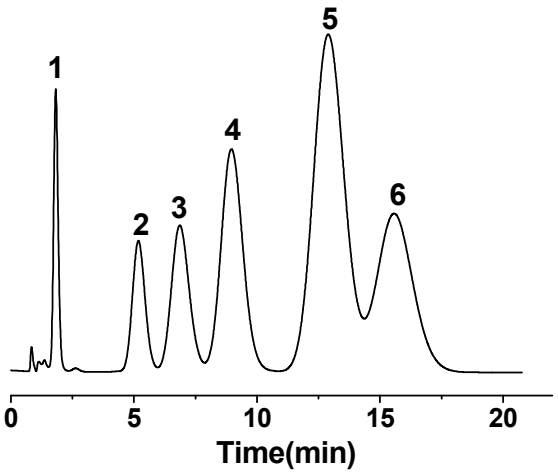

Fig.7 HPLC chromatograms on the MIL-53(Fe) packed column for the separation of : (1) naphthalene;(2) acenaphthylene;(3) benzo(k)fluoranthene;(4) anthracene;(5) fluorene;(6) phenanthrene using 100% ACN as the mobile phase at a flow rate of 0.4 mL min^{-1}. All the separations were performed at room temperature and monitored with a UV detector at 254 nm.

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